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Effect of Sunlight on Enumeration of Indicator Bacteria Under Field Conditions†

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The effect of sunlight on the enumeration of fecal coliform (FC) and fecal streptococcal (FS) bacteria when water samples are collected in containers and brought back to the laboratory for analysis or when the water samples are filtered through membranes on site was determined. FC and FS in raw sewage stored in clear glass or translucent polyethylene containers were resistant to the effects of sunlight. However, under the same conditions of storage and exposure to sunlight, 90% of FC and FS in sewage diluted 1:100 in seawater were inactivated within 13 to 32 min. When sewage was similarly diluted in stream water and exposed to sunlight, 90% of FC were inactivated after 28 to 38 min, whereas 90% of FS were not inactivated even after a 2-h exposure to sunlight. Other experiments showed that 90 to 99% of FC and FS retained on membranes were inactivated when these membranes were exposed to sunlight for 10 to 15 min. FS were inherently more resistant to sunlight inactivation than were FC. Finally, evidence was obtained to show that sunlight initially stresses the bacteria but eventually causes cell death.

The hygienic quality of environmental waters is usually assessed by analyzing water samples for concentrations of indicator bacteria such as fecal coliforms (FC) or fecal streptococci (FS). Sampling of waters is usually conducted during daylight hours and in the presence of sunlight. In a recent study (9), we reported that sunlight is the primary factor controlling the survival of indicator bacteria suspended in water under field conditions and that 90% of sewage-borne FC and FS suspended in seawater at 15 to 25°C can be inactivated within 30 and 60 min, respectively, after exposure to sunlight. Furthermore, the bactericidal effect of sunlight is able to penetrate clear glass, translucent polyethylene, and 3.3 m of clear seawater. Convincing evidence that sunlight can inactivate coliform bacteria suspended in seawater was first reported by Gameson and Saxon in 1967 (10). However, due in part to many other reports which have claimed that other factors such as high salinity (5), heavy metals (11), sedimentation-flocculation (15), nutrient deficiency (5), predation by other microorganisms (8), lysis by bacteriophage (6), or presence of microbial toxins (2) are involved in inactivation, the role of sunlight as an effective natural means of inactivating enteric bacteria in environmental waters has not been widely accepted. However, after reviewing all of the published data, Chamberlin and Mitchell in 1978 (7) concluded that under field conditions sunlight is probably the most effective bactericidal factor in natural waters. Additional evidence for the bactericidal effect of sunlight has been reported by Bellair et al. (3) and by McCambridge and McMeekin (14). If sunlight is such an effective bactericidal agent, it could inactivate a significant fraction of the indicator bacteria in water samples being processed or transported under field conditions. Significantly, the possible bactericidal effect of sunlight is not mentioned and specific recommendations to prevent exposure of water samples to sunlight are not given in water quality manuals, including *Standard Methods for Examination of Water and Wastewater* (1). It should be noted that due to common problems such as great distances between the sampling sites and the laboratory or vehicle, as well as the practice of collecting multiple samples during a single day, it is conceivable that water samples may be exposed to sunlight for up to 30 min. The purpose of this study was to determine the effect of sunlight on the enumeration of FC and FS when water samples are obtained under field conditions and analyzed by the membrane filtration method.

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MATERIALS AND METHODS

Source of sewage, seawater, and freshwater. Seawater was obtained from Black Point Beach, located on

the southeastern coast of Oahu, Hawaii, at a depth of 1.2 m. This beach does not receive stream or storm drain runoff and is not extensively used for swimming. Freshwater was obtained from Nuuanu Stream at an elevation of 800 ft (ca. 236.4 m) above sea level close to a forest reserve area to ensure that the water was not significantly contaminated with domestic wastes. Raw sewage was obtained from the Ala Moana Pump Station, which collects the sewage from a major portion of the city of Honolulu, Hawaii. In an effort to study the maximum effect of sunlight on wastewater, samples of sewage were clarified of large particulate matter by filtration through an AP 25 prefilter (Millipore Corp.). It should be noted that prefiltering samples will result in unpredictable percent removals of organisms and should not be done in routine monitoring analyses.

Bacteriological analysis. The standard membrane filtration method (1) was routinely used. Briefly, 25 ml of the water sample was filtered through 47-mm membranes of 0.45-μm porosity (Gelman Sciences, Inc.), and the filters were placed on mFC agar (Difco Laboratories) without rosolic acid for selective recovery of FC at 44.5 ± 1°C or on KF agar (Difco) for selective recovery of FS at 35 ± 1°C. For resuscitation studies, the procedure described by Lin (13) and cited as one of the acceptable methods in *Standard Methods for Examination of Water and Wastewater* (1) was used. Briefly, the membranes through which the water samples were filtered were placed on phenol red lactose agar (PRLA) and incubated for 4 h at 35°C before they were transferred onto selective media for incubation at 44.5 or 35°C for the specific recovery of FC and FS, respectively.

Experimental design. Two experimental designs were developed to determine the effect of sunlight on the analyses of field water samples. The first design was used to evaluate the effect of sunlight when field water samples are collected in clear glass or polyethylene containers and transported back to the laboratory for processing. For this protocol, water samples were placed into either 8-ounce (ca. 240-ml) clear soda-lime glass bottles (Brockway Glass Co.) or 500-ml translucent linear (high-density) polyethylene bottles (Nalgene Labware Department, Sybron/Nalge). One set of these bottles was exposed to sunlight with the bottles on their sides on a plastic tray, whereas another set of bottles was shielded from sunlight by either wrapping the bottles with a black trash bag liner or storing the bottles in the laboratory. The concentrations of FC and FS in these samples after various time periods were determined by the membrane filtration method. The second design was used to evaluate the effect of sunlight when water samples are processed on site by filtering water samples through membranes and placing the membranes on selective media prepared in disposable polystyrene petri dishes. One set of dishes was placed on plastic trays and exposed to sunlight, whereas a second set was shielded from sunlight as described above. After various time periods, the concentrations of FC and FS on the membranes were determined by incubating the mFC agar plates at 44.5°C and KF agar plates at 35°C. Experiments in which the samples were exposed to sunlight were conducted on the roof of Holmes Hall at the University of Hawaii, Honolulu. In these experiments, the quantity and quality of sunlight as well as the chemical quality of the environmental samples could not be controlled and varied from day to day. To minimize these variations, experiments were conducted only between the hours of 1000 and 1500 and only on predominantly sunny days when the measurements of visible light with the LI-192S quantum sensor (Lamda Instruments Corp.) ranged between 2.4×10^6 and 1.5×10^7 microeinsteins per m^2 per h. Moreover, samples of sewage, seawater, and stream water were obtained on the day of the experiment. Despite these precautions, it was observed that the T_{90} (the time required for 90% of the population of FC or FS to be inactivated) varied to some degree when the same experiment was conducted on different days. However, the inactivation rates of FC and FS relative to each other and to the suspending medium were consistent from experiment to experiment. Consequently, the data from each experiment were considered to be unique to that set of experimental conditions, and results of similar experiments conducted on different days were not averaged. Instead, experiments were repeated a minimum of three times on three separate days, and conclusions were drawn only after data from the three experiments were consistent with each other. Within each experiment, replications of two analyses per sampling point were averaged. The rates of bacterial inactivation under the various conditions were compared by calculating the respective T_{90}s.

RESULTS

Inactivation of indicator bacteria in storage containers. The collection of field water samples in clear glass or translucent polyethylene containers for transportation back to the laboratory is a commonly used procedure. Although there is a general recommendation that all samples should be immediately chilled and shielded from sunlight, this precaution is not vigorously emphasized, and samples are often exposed to sunlight for periods of 5 to 30 min before they are properly stored. To determine whether sunlight can penetrate containers to inactivate FC and FS within the sample containers, 250-ml clear glass bottles or 500-ml translucent linear polyethylene bottles were filled with 100% raw sewage (10^6 and 10^5 per 100 ml for FC and FS, respectively) or with sewage diluted 1:100 in clear seawater or clear mountain stream water. The two types of containers filled with the three types of samples were then stored either in the presence of bright sunlight or totally shielded from sunlight. After 5, 10, 15, 20, 30, 60, and 120 min of storage, representative bottles were immediately assayed for concentrations of FC and FS by the standard membrane filtration method. The concentrations of FC and FS in all of the samples maintained in the laboratory in the absence of sunlight at 24 ± 2°C were stable over the 2-h incubation period (i.e., the concentrations did not increase or decrease by more than 25%). However, in the presence of sunlight, the T_{90}s of FC in the sewage-seawater mixture

stored in glass containers and in translucent polyethylene containers were only 13 and 17 min, respectively, whereas the T_{90}s of FS in the same samples were 26 and 32 min, respectively. Under the same sunlight conditions in the sewage-stream water mixture, the T_{90}s for FC in samples stored in the glass and polyethylene containers were extended to 28 and 38 min, respectively, whereas T_{90}s for FS in the same two containers were not achieved after a 2-h exposure to sunlight. In contrast, T_{90}s for both FC and FS in undiluted sewage were not achieved in either container after a 2-h exposure to sunlight. It should be noted that in this experiment, the same sewage, seawater, and freshwater samples were used throughout, and the entire experiment was conducted under the same sunlight conditions. When the experiment was repeated on different days with different samples of sewage, seawater, and freshwater, variation in the T_{90} was observed, reflecting variation in the samples as well as in the sunlight conditions. However, the results followed the same pattern and consistently indicated that sunlight can penetrate both clear containers and translucent polyethylene containers to inactivate FC and FS in dilute water samples. Moreover, FC and FS in sewage-freshwater medium were more resistant to the bactericidal effect of sunlight than were FC and FS in the sewage-seawater medium. For example, when the experiment described above was conducted on another day, the T_{90}s for FC and FS in the sewage-seawater mixture stored in glass bottles and exposed to sunlight were 13 and 59 min, respectively. Under the same sunlight conditions, the T_{90}s for FC and FS suspended in the sewage-freshwater sample were 116 and 109 min, respectively. As demonstrated by these results, greater variation in survival of FC and FS was observed when sewage was mixed with different samples of freshwater and exposed to sunlight compared with sewage mixed with different seawater samples and exposed to sunlight.

One unavoidable consequence of exposing water samples to sunlight is the warming of the sample. Thus, the temperature of the sample in glass bottles rose to maximums of 35 and 45°C after storage in sunlight for 30 min and 2 h, respectively. To determine whether the high temperature (35 to 45°C) is a requirement for the inactivation of FC and FS by sunlight, sewage samples diluted 1:100 with seawater were added to polyethylene bottles, and one set of bottles was placed on trays and exposed to sunlight as described above. Another set of these bottles exposed to sunlight was partially submerged in ice water to maintain the temperature of the samples at 15 to 20°C even after 1 h of exposure to sunlight. The T_{90}s for FC and FS in the bottles placed on the tray were 22 and 48 min, respectively, whereas the T_{90}s for FC and FS in the bottles bathed in ice water were 28 and 48 min, respectively. These results indicate that sunlight inactivation of FC and FS is not dependent on the warming of the suspending medium to 35 to 45°C and can be expected to occur even when the temperature of the water is maintained at 15 to 20°C.

Stressful versus bactericidal effect of sunlight. One unresolved question appeared to be whether the observed inactivation of indicator bacteria by sunlight represents true die-off or stress. As described by Bissonnette et al. (4), stressed or sublethally injured organisms are unable to grow on selective media but remain a part of the total viable population, as they are capable of producing colonies on a rich, nonselective medium. It is therefore important that stressed organisms be identified to correctly evaluate the effects of sunlight. To address this question, a 1:100 dilution of sewage in seawater was placed in a 2-liter beaker, exposed to sunlight for various time periods, and analyzed for FC and FS by the standard membrane filtration method (1) and by the two-step resuscitation technique described by Lin (13). The populations of FC and FS in the sewage-seawater sample stored in the absence of sunlight remained essentially unchanged over the 4-h period whether assayed by the standard method or the resuscitation method (Table 1). However, when the sample was exposed to sunlight, the recoveries of FC and FS assayed by the resuscitation method were generally higher than when the direct standard membrane filtration method was used. Significantly, the resuscitation method was able to recover most of the FC and FS in samples exposed to sunlight for 15 to 30 min, whereas inactivation of approximately 90% of FC and FS was observed after this amount of time when assayed by the standard method. However, after 1- and 2-h exposures to sunlight, most of the FC and FS measured as inactivated by the standard method could not be recovered by the resuscitation method. Moreover, after a 3-h exposure to sunlight, the concentrations of FC and FS in the water sample were determined to be less than 1 colony-forming unit per 25 ml whether the standard method or the resuscitation method was used. These results indicate that sunlight initially causes sublethal injury to the bacteria but that after continuous reaction, the bacteria are irreversibly inactivated.

Inactivation of indicator bacteria on membranes. To more accurately determine the concentration of bacteria in the environment, it is recommended that water samples be processed on site rather than be transported back to the

TABLE 1. Comparison of standard and resuscitation methods in the recovery of FC and FS from a sewage-seawater mixture exposed to sunlight[a]

Bacteria	Assay method	No. of bacteria per 25 ml after no. of h in:						
		Absence of sunlight		Presence of sunlight				
		0	4	0.25	0.5	1.0	2.0	3.0
FC	Standard	1.9×10^4	1.1×10^4	7.0×10^3	1.6×10^3	6.4×10^1	<1.0	<1.0
	Resuscitation	2.3×10^4	1.9×10^4	2.6×10^4	1.1×10^4	3.9×10^2	1.0	<1.0
FS	Standard	1.1×10^3	1.1×10^3	9.8×10^2	2.8×10^2	8.1×10^1	<1.0	<1.0
	Resuscitation	1.1×10^3	1.5×10^3	1.5×10^3	6.0×10^2	1.3×10^2	1.0	<1.0

[a] A 1:100 dilution of sewage in seawater was placed into two 2-liter beakers; one beaker was incubated in the laboratory at 24 ± 2°C in the absence of sunlight, whereas the other beaker was incubated in the presence of sunlight within a water jacket to maintain the water temperature at 24 ± 2°C. After various periods of storage, samples were taken and assayed for FC and FS by the standard and resuscitation methods.

laboratory for processing. On-site processing involves filtering a water sample through a membrane and placing the membrane on a selective medium for growth of the bacteria. Although this technique precludes the problems involved in transporting the water samples back to the laboratory, it requires more manipulations, apparatus, and time at each of the sampling sites. Moreover, since the membranes must then be transported back to the vehicle or laboratory, they may also be exposed to sunlight before being properly stored. To determine the effect of sunlight on the concentrations of FC and FS which had been retained on the membranes, 25-ml aliquots of a 1:100 dilution of sewage in seawater were filtered through membranes which were then placed on disposable polystyrene petri dishes containing either mFC agar to recover FC or KF agar to recover FS. These dishes were covered with their polystyrene lids and individually placed on a tray with the surface of the membranes facing the sun. After 5, 10, 15, 20, and 30 min of exposure to sunlight, representative dishes were taken back to the laboratory where they were immediately incubated at their selective temperatures for the enumeration of FC and FS. The colony counts of FC and FS on the membranes were only slightly reduced after 5 min of exposure to sunlight (Fig. 1). However, after 10 and 15 min of exposure to sunlight, the concentration of FC was reduced by 99% and nearly 99.9%, respectively, whereas the concentration of FS was reduced by nearly 90 and 99%, respectively. After 20 min of exposure to sunlight, no colonies of either FC or FS appeared on the membranes. Similar results were obtained whether the original samples were undiluted raw sewage or sewage diluted 1:100 into fresh stream water, indicating that the nature of the original suspending medium plays a minor role after the medium is filtered through the membrane and the indicator bacteria are retained on the membrane and exposed to sunlight. The concentrations of FC and FS on membranes not exposed to sunlight (controls) remained stable whether the membranes were immediately incubated or held for 30 min at

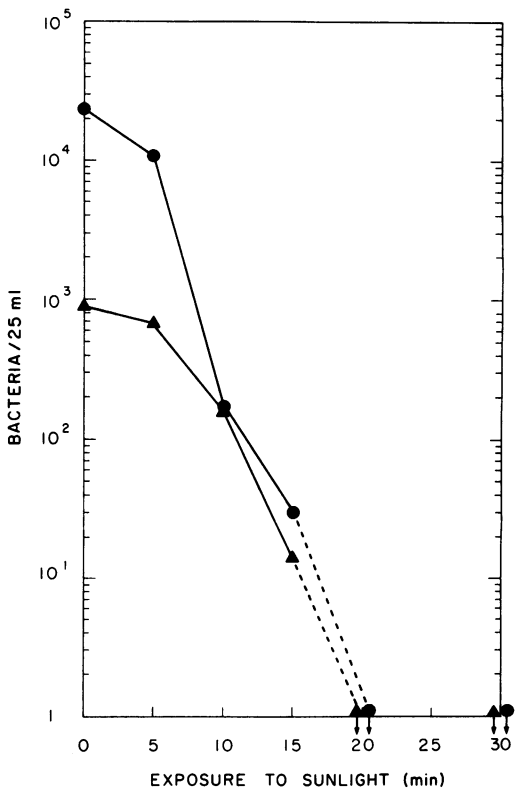

FIG. 1. Effect of sunlight on survival of FC and FS retained on membranes and enumerated by the standard membrane filtration method. Membranes for recovering FC (●) were placed on mFC agar; membranes for recovering FS (▲) were placed on KF agar.

room temperature (24°C) before being incubated.

Two obvious consequences of exposing membranes on media to sunlight were the warming of the agar medium and the formation of water droplets (condensate) on the underside of the petri dish lid. To assess these two effects on sunlight inactivation of indicator bacteria retained on the membranes, 25-ml aliquots of sewage diluted 1:100 in seawater were filtered through membranes which were then placed on mFC or KF agar. One set of these membranes in petri dishes was placed on a tray and exposed to sunlight as was done previously, whereas a second set of petri dishes was placed on a layer of crushed ice and exposed to the same sunlight conditions. After 5, 10, 15, 20, and 30 min of exposure to sunlight, representative dishes from both sets were removed from sunlight and immediately incubated (mFC agar at 44.5°C and KF agar at 35°C). Condensation was visually assessed and was scored as 1+ when the condensate was small and well spaced and covered approximately 25% of the underside surface of the petri dish lid. As the medium warmed, the condensate coalesced, forming larger water droplets effectively covering 50 (2+), 75 (3+), and 100% (4+) of the surface area of the petri dish lid. The temperature of the agar medium was measured with a thermometer stuck into the agar after the 30-min exposure to sunlight. For petri dishes placed directly on a tray, a 1+ condensation was observed after 5 min of exposure to sunlight and increased to a 4+ condensation by 15 min. Moreover, the temperature of the agar increased to 30°C after a 30-min exposure to sunlight. On the other hand, placing the petri dishes on ice resulted in only a 1+ maximum condensation and an agar temperature of 25°C after a 30-min exposure to sunlight. Despite marked differences in the agar temperature and the amount of condensation in the two sets of petri dishes, the T_{90}s for FC in the two sets were 4.5 and 4.3 min, respectively, whereas the T_{90}s for FS in the same two sets were 10.0 and 9.5 min, respectively. These results indicate that cooling of the agar or formation of condensation does not effectively inhibit the bactericidal action of sunlight.

As with indicator bacteria suspended in a liquid medium, the reduction of bacterial colonies due to exposure of the membranes to sunlight may represent stress or irreversible inactivation. To assess this possibility, 25-ml aliquots of a 1:100 dilution of sewage in seawater were filtered through membranes which were then placed on PRLA, mFC agar, or KF agar. These petri dishes were then placed on trays and exposed to sunlight for 5, 10, 15, 20, and 30 min before representative dishes were immediately removed from sunlight and incubated (mFC agar at 44.5°C and KF agar at 35°C). On the other hand, membranes on PRLA were incubated for 4 h at 35°C before they were transferred onto mFC agar for incubation at 44.5°C or onto KF agar for incubation at 35°C. Although the resuscitation method consistently recovered more colonies of FC than did the standard method, the kinetics of FC inactivation resulting from exposure of the membranes to sunlight were similar in both assay techniques (Fig. 2A). Inactivation of 90 and 99.9% of the FC on the membranes was observed after 10 and 20 min of exposure to sunlight, respectively, whether the membranes were placed on PRLA or on mFC agar. Furthermore, after 30 min of exposure to sunlight, no colonies of FC were detected on the membranes whether the standard method or the resuscitation method was used. On the other hand, after 10 and 20 min of exposure to sunlight, the colonies of FS on the membranes placed on KF agar were reduced by greater than 90 and 99.9%, respectively. However, the concentration of FS on the membranes placed on PRLA and assayed by the resuscitation method remained unchanged even after 30 min of exposure to sunlight. These results indicate that the resuscitation technique was effective in recovering the populations of FS which were measured as inactivated when the standard technique was used. On the other hand, the resuscitation technique was not able to recover the populations of FC which were measured as inactivated when the standard technique was used.

DISCUSSION

There is mounting evidence (7, 9, 10, 12, 14) that sunlight is bactericidal and plays an important role in controlling the survival of indicator bacteria in natural waters. This investigation provides evidence that under anticipated field conditions of sampling environmental waters, exposing samples to sunlight may result in the reduction of the original concentrations of FC and FS in the samples. Environmental water samples are often collected in clear glass or translucent polyethylene containers, and these samples are transported back to the laboratory where they are analyzed for concentrations of FC and FS. The possibility that these water samples may be exposed to sunlight for periods of 15 to 30 min or more is not uncommon, especially since standard water quality manuals do not specifically caution against this practice. The bactericidal effect of sunlight was shown to be able to penetrate both clear glass and linear polyethylene containers, resulting in the inactivation of 90% of the populations of FC in a 1:100 mixture of sewage and seawater after 13 to 17

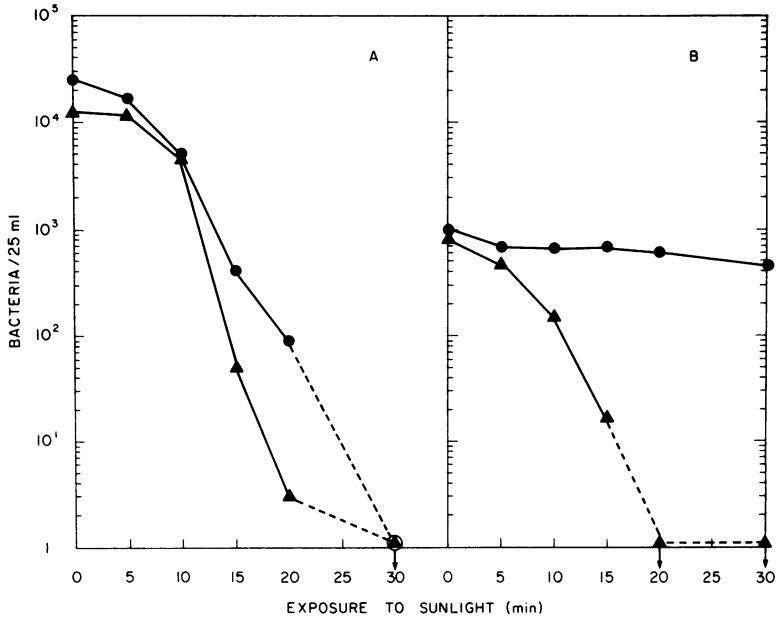

FIG. 2. Effect of sunlight on survival of FC and FS retained on membranes and placed on selective medium (mFC or KF agar) for enumeration by the standard filtration method versus that of FC and FS retained on membranes placed on nonselective medium (PRLA) and enumerated by the resuscitation method. (A) Recovery of FC when membranes were placed on mFC agar (▲) or PRLA (●) and exposed to sunlight. (B) Recovery of FS when membranes were placed on KF agar (▲) or PRLA (●) and exposed to sunlight.

min of exposure to sunlight. Under the same sunlight conditions, 90% of the populations of FS in the same samples were inactivated after 26 to 32 min of exposure to sunlight. Inactivation of indicator bacteria was only slightly retarded when water samples were stored in translucent polyethylene containers rather than in clear glass containers. Consequently, the common assumption that translucent polyethylene containers block out the detrimental effects of sunlight is not valid. It should be noted that the rapid sunlight-induced inactivation of FC and FS in the 1:100 sewage-seawater mixture was substantially retarded when sewage was diluted 1:100 into clear, fresh, stream water and was completely retarded when undiluted sewage was exposed to the same sunlight conditions. Undoubtedly, the high turbidity and high organic content of undiluted sewage protected the bacteria from the inactivating effect of sunlight. The basis for the relative stability of indicator bacteria when sewage was diluted into stream water remains uncertain and is currently being investigated.

Field water samples may also be filtered through membranes at the sampling site, and the membranes may be placed directly on mFC or KF agar medium for the growth and enumeration of FC and FS, respectively. When this technique is used, the membranes retaining the indicator bacteria, rather than the water sample, may be exposed to sunlight during transportation back to the laboratory. After 10 min of exposure to sunlight, 90% of FC and FS on these membranes placed on media and housed within disposable polystyrene petri dishes were inactivated. These results indicate that FC and FS, whether suspended in liquid medium or retained on membranes, are susceptible to the inactivating effect of sunlight. FS were more resistant to sunlight inactivation than were FC, under both conditions, indicating that FS are inherently more resistant to sunlight inactivation than are FC.

The standard membrane filtration method was used throughout this study to determine the effect of sunlight on bacteria. However this method simply measures the loss of bacterial colony-forming capacity and does not differentiate stressed bacteria, which are able to grow on nutrient-rich medium but not on selective medium, from killed bacteria. To determine whether sunlight stressed or killed the bacteria, water samples and membranes containing FC and FS were exposed to sunlight, and the concentrations of the two bacteria were compared by using the standard membrane filtration method and the two-step resuscitation method of Lin

(13). The results indicated that in the aqueous environment, sunlight initially stresses without killing the indicator bacteria, as reported by Kapuscinski and Mitchell (12). However, after 10 to 20 min of exposure to sunlight, most of the bacteria could not be rescued by the resuscitation method, indicating that cell death had occurred. This same conclusion was reached in our initial study (9), in which we used the one-step resuscitation technique of Rose et al. (16). Similar results were obtained when membranes rather than water samples were exposed to sunlight, and FC were assessed by using the standard membrane filtration method and the resuscitation method of Lin (13). However, when the membranes were exposed to sunlight and assayed for FS, rapid inactivation of FS was observed when the standard membrane filtration method was used, whereas no inactivation of FS was observed when the resuscitation method was used. One interpretation of these findings is that FS undergo a longer period of stress than do FC and that stressed bacteria can be rapidly resuscitated when favorable growth conditions are immediately present.

In conclusion, the results of this study indicate that indicator bacteria, whether suspended in water or retained on membranes, are susceptible to inactivation by sunlight. Moreover, the bactericidal effect of sunlight can be expected to penetrate clear glass and clear polystyrene as well as translucent polyethylene. Consequently, the common assumption that translucent polyethylene containers protect samples from the harmful effects of sunlight does not appear to be valid. Certainly, the common practice of leaving water samples uncovered in the back of pickup trucks should be discouraged. Finally, in the extrapolation of the results of this study, it should be emphasized that the clear seawater and freshwater used in this study as well as the clear atmosphere and geographical latitude of the Hawaiian Islands probably reflect ideal conditions for sunlight inactivation of bacteria. Although these conditions are not unique to the Hawaiian Islands, it should be recognized that atmospheric conditions, water quality, and geographical latitude are variables which may moderate the bactericidal effectiveness of sunlight in different areas of the world. Nevertheless, as a result of this study, we recommend that personnel required to collect environmental water samples be informed of the possible bactericidal effects of sunlight and be instructed to take proper precautions, such as the shielding of field samples from exposure to sunlight. Since dark brown glass and dark brown polyethylene containers are able to block out the bactericidal effects of sunlight, these kinds of sample containers are highly recommended.

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